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Possible Neurologic Effects of Aspartame, a Widely Used Food Additive

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The artificial sweetener aspartame (L-aspartyl-L-phenylalanyl-methyl ester), is consumed, primarily in beverages, by a very large number of Americans, causing significant elevations in plasma and, probably, brain phenylalanine levels. Anecdotal reports suggest that some people suffer neurologic or behavioral reactions in association with aspartame consumption. Since phenylalanine can be neurotoxic and can affect the synthesis of inhibitory monoamine neurotransmitters, the phenylalanine in aspartame could conceiveably mediate neurologic effects. If mice are given aspartame in doses that elevate plasma phenylalanine levels more than those of tyrosine (which probably occurs after any aspartame dose in humans), the frequency of seizures following the administration of an epileptogenic drug, pentylenetetrazole, is enhanced. This effect is simulated by equimolar phenylalanine and blocked by concurrent administration of valine, which blocks phenylalanine's entry into the brain. Aspartame also potentiates the induction of seizures by inhaled fluorothyl or by electroconvulsive shock. Perhaps regulations concerning the sale of food additives should be modified to require the reporting of adverse reactions and the continuing conduct of mandated safety research.

Introduction: Food Additives as Neuroactive Environmental Constituents

For the very great majority of Americans, i.e., those who elect to eat processed foods, food additives are a ubiquitous constituent of the environment, and one with potentially important health effects. The laws governing the sale of these compounds require that their addition to foods fulfill a specific purpose, such as improving flavor, retarding spoilage, or enhancing nutritional quality, and that such use be risk-free. Implicit in this latter requirement is the expectation that the food additive not be found to affect physiological processes other than the nutritional or sensory ones underlying its use: Compounds that do affect physiological systems are classified as drugs by the Food and Drug Administration (FDA), and are subject to considerably more demanding regulatory procedures than food constituents. Moreover, because food additives must be shown to be physiologically inert in order to win initial FDA approval, once they have obtained this approval they are exempted from the requirement, imposed on all drugs, that their safety be continuously monitored: Companies that manufacture and use approved food additives are not obligated to monitor adverse reactions associated with consumption of their product, nor to submit to the FDA reports of such adverse reactions; they also are not required to carry out further government-mandated research programs to affirm their product's safety.

However, the consumption of a number of food additives can cause physiological effects which include, for some, modification of the chemical composition and functional activities of the nervous system (*1,2*). These effects may generate health risks for some people. Moreover, in the case of one such compound, the artificial sweetener aspartame (L-aspartyl-L-phenylalanyl-methyl ester), these neural effects were largely unexplored prior to the compound's addition to the food supply, and were not a factor in calculating the quantities that individuals can safely consume (the ADI, or acceptable daily intake, currently set for aspartame at 50 mg/kg) (*3*). The effects of aspartame, and of certain other food additives, like caffeine, on the nervous system are sometimes not of such a nature as to allow their detection by the standard neurotoxicological tests used to assess the safety of food additives, inasmuch as these effects need not be associated with cell death, nor with other visible manifestations of neuronal damage. Rather, they involve more subtle biochemical changes, as well as functional consequences that are demonstrable only in specially treated animals (*4*) (and possibly, by extrapolation, only in especially vulnerable people).

Although these physiological effects are unrelated to the reason that the additive was placed in the food, they may have important health implications just the same, given the very large number of Americans who are routinely exposed to environmental constituents added to

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the food supply. If only 1% of the 100,000,000 Americans thought to consume aspartame ever exceed the sweetener's ADI, and if only 1% of this group happen coincidentally to have an underlying disease that makes their brains vulnerable to the effects of an aspartame-induced rise in brain phenylalanine levels, then the number of people who might manifest adverse brain reactions attributable to aspartame would still be about 10,000, a number on the same order as the number of neurally related consumer complaints already registered with the FDA and other federal agencies (*5,6*).

This report describes some of the available evidence, almost all of which has been accumulated in the past 2 years, that doses of aspartame which are within the range actually consumed by some people can affect the chemical composition of the brain, and may thereby contribute to particular CNS side effects, including headaches (*7*), inappropriate behavior responses (*8,9*), and seizures (*10,11*). As will be noted, progress in anticipating aspartame's effects on the human brain has been hampered by a particular experimental problem related to differences in the speeds at which the rodent and human livers metabolize phenylalanine, the lone neutral amino acid in aspartame, to tyrosine. The major biochemical effect of aspartame, in humans, is to raise blood and, presumably, brain phenylalanine levels (*12*); in contrast, its main effect in rodents is to raise blood (and brain) tyrosine levels (*13,14*), and tyrosine is often the antidote to phenylalanine's effects on the brain. This species difference (which makes questionable the extrapolation of much of the rodent literature to humans) then can be circumvented by using the rats or mice only as a source of tissues for *in vitro* studies, or by administering the aspartame in doses that transiently overwhelm the animals' capacity to metabolize it so that, as happens when people consume any dose, the sweetener causes brain phenylalanine to rise proportionately more than brain tyrosine.

The existence of this major metabolic difference between rodents and people underscores the necessity that large-scale human studies be carried out, preferably on selected populations whose members might be especially vulnerable to phenylalanine and to aspartame's other breakdown products, before conclusions be drawn about whether or not aspartame really is risk-free. If aspartame cannot be shown to be risk-free, perhaps its regulatory classification could be changed; for example, to that of over-the-counter drug. Or perhaps the federal regulation of novel food additives should be modified to require that adverse reactions be monitored and reported, and that continuing research on their safety be carried out as mandated by the FDA.

Effects of Dietary Aspartame on Brain Phenylalanine Levels: Possible Consequences for Neurotransmission

The consumption of an aspartame-laden food or beverage contributes to the plasma the three natural compounds contained within the aspartame molecule: the amino acids phenylalanine and aspartic acid, and the alcohol methanol (*15*), possibly as well as various peptides (like β-aspartame or the aspartyl-phenylalanine diketopiperazine) that are formed from it spontaneously, on the shelf, or enzymatically, after its consumption. Our present concern is about the CNS effects of the phenylalanine. The aspartic acid is unlikely to cross the blood-brain barrier (*4*), and very few data are available showing that the amounts of methanol or peptides generated by ADI doses of aspartame have significant neural effects. Underlying our concern about the possible brain effects of the phenylalanine in ADI aspartame doses (approximately 2 g in a 175 lb man) are the following relationships:

Plasma phenylalanine levels are not regulated by any known homeostatic mechanism. At any particular time plasma levels simply reflect the amounts of phenylalanine being absorbed from the foods most recently eaten (*16,17*). Thus, phenylalanine levels can normally vary between 30 and 90 μM, depending upon whether the subject has most recently eaten no-protein (i.e., phenylalanine-free) or high-protein meals. Consumption of the ADI aspartame dose is thus able to elevate plasma phenylalanine levels about threefold (*18*).

Consumption of dietary phenylalanine in the usual way, as a constituent of protein, does not elevate brain phenylalanine levels (*19*). This is because the protein elevates plasma levels of the other large neutral amino acids (LNAA) (valine, leucine, isoleucine, tryptophan, tyrosine) more than those of phenylalanine. These other amino acids are considerably more abundant than phenylalanine in the protein, and the branched-chain amino acids, unlike phenylalanine, are largely unmetabolized when they pass through the portal circulation (*20*).

In contrast, consumption of phenylalanine in the form of aspartame, with the other LNAA, that are always present in proteins, elevates plasma phenylalanine levels without elevating those of the other LNAA, this causes marked elevations in the plasma phenylalanine ratio (the ratio of the plasma phenylalanine concentration to the summed concentrations of the other LNAA) (*13*). It should be noted that aspartame is probably the only phenylalanine-containing food that man has ever eaten which elevates this ratio.

An elevation in the plasma phenylalanine ratio causes a parallel rise in brain phenylalanine levels, since a single transport macromolecule within the endothelial cells lining the brain's capillaries mediates the uptake of all of the LNAA; this macromolecule is unsaturated at normal plasma LNAA levels; and each of the LNAA's compete for attachment to it, their success depending on their relative affinities for it and their plasma concentration relative to those of its competitor (*4,21*). The elevation in the plasma phenylalanine ratio also tends to reduce the corresponding ratios for the other LNAA, thus decreasing their brain uptakes and tending to lower their brain levels (*13*). [Aspartame fails to lower brain tyrosine levels in the rat because the rat's liver hydroxylates dietary phenylalanine so rapidly that plasma

tyrosine levels rise even more than those of plasma phenylalanine (*13,14*). However, in humans dietary aspartame probably reduces brain tyrosine uptake, depending on the dose consumed.]

If an aspartame-containing beverage is consumed along with, for example, a carbohydrate-rich, protein-poor dessert food, its effects on brain phenylalanine are doubled (*13*). This is because the insulin secretion elicited by the carbohydrate selectively lowers plasma levels of the branched-chain amino acids (by facilitating their uptake into skeletal muscle), without having much of an effect on plasma phenylalanine; this increases the effect of the aspartame on the plasma phenylalanine ratio (*17*). A similar doubling may occur if the eater happens to be one of the perhaps 10 million Americans (*22*) who are, without knowing it, heterozygous for the phenylketonuria (PKU) gene.

Once within brain, neurons producing certain neurotransmitters, such as dopaminergic nigrostriatal cells, the excess phenylalanine can inhibit enzymes (like tyrosine hydroxylase) needed to synthesize the neurotransmitters. Excess circulating phenylalanine can also diminish the production of brain catecholamines and serotonin by competing with their precursor amino acids for transport across the blood-brain barrier. Hence, physiological processes that depend on the sustained release of adequate quantities of these transmitters can be affected.

One such process, in rodents, is the suppression of seizure activity. It has been recognized for years that animals given drugs [such as reserpine or Ro 4-1284 (*23*)] that deplete the brain of particular monoamine neurotransmitters, or that block the receptor-mediated effects of these transmitters, exhibit greater sensitivity to seizures (*23*). In contrast, drugs [such as L-Dopa plus an MAO inhibitor, or L-Dops (*23*)] thought to enhance monoaminergic neurotransmission apparently protect rodents against the development of seizures. Low doses of aspartame, which raise plasma tyrosine levels more than those of phenylalanine, might be expected to have no effect on seizure thresholds, or even to protect animals against the epileptogenic effects of drugs like pentylenetetrazole; in contrast, comparable doses, given to humans, could enhance seizure susceptibility, since, in humans, all aspartame doses apparently cause greater increases in plasma (and brain) phenylalanine than in tyrosine. (As shown below, sufficiently high aspartame doses, which transiently exceed the liver's capacity to hydroxylate phenylalanine, can also potentiate seizures in rodents, whether these seizures are generated by drugs, electroshock, or inhalation of fluorothyl.)

All of these relationships have now been demonstrated; most recently, the ability of phenylalanine to suppress dopamine release from the rat's brain has been demonstrated. Slices of caudate nucleus were superfused with a solution containing sufficient tyrosine (50 μM) to sustain dopamine's release, and were stimulated electrically [360 pulses; 12 Hz; 2 msec (*24*)] on two occasions, separated by an interval of about 60 min. The addition of phenylalanine to the medium caused a dose-related suppresion of subsequent dopamine release (shown as a reduction in the S2/S1 ratio). The lowest phenylalanine concentration capable of impairing dopamine release (200 μM) was about three times that present in plasmas from fasting rats. An aspartame dose that causes a proportionate threefold rise in the human phenylalanine content of plasma is the ADI dose (50 mg/kg). As explained above, this dose probably falls to 25 mg/kg if the aspartame is consumed along with a dietary carbohydrate (*13*), or to 12.5 mg/kg if the person consuming it in this manner also happens to be heterozygous for PKU.

Effects of Aspartame on Seizure Susceptibility in the Mouse

To determine whether aspartame intake could modify seizure susceptibility, perhaps by increasing plasma and brain phenylalanine levels, one of our group has examined its effects on the incidence of seizures, their speed of onset, and the amount of convulsant required to produce the seizures among mice given treatments known to be epileptogenic (*25*). In general, animals received various aspartame doses 1 hr before a CD_{50} dose of the seizure-inducing treatment, or a fixed aspartame dose 1 hr before various doses of the treatment. The number of animals in each treatment group exhibiting seizures in the next 60 min were counted (when the treatment was pentylenetetrazole), or the time passing

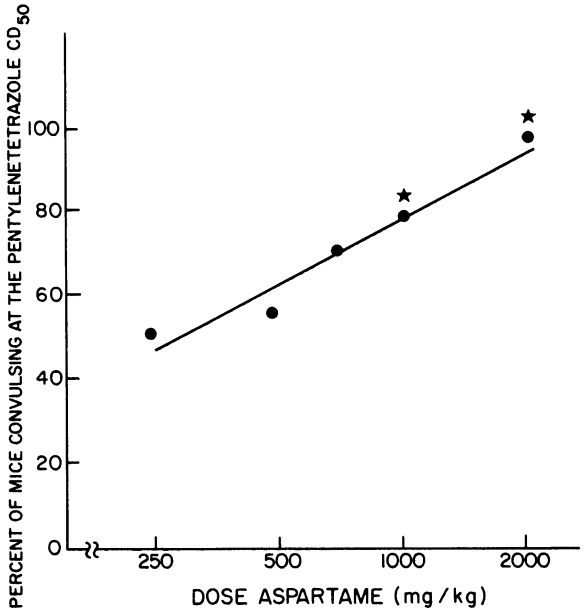

FIGURE 1. Effect of aspartame pretreatment on the percentage of mice convulsing following the administration of the CD_{50} dose of pentylenetetrazole. Groups of male CD-1 mice (average $n = 24$) received 0–2000 mg/kg aspartame via oral intubation followed by an SC injection of pentylenetetrazole 1 hr later. The number of mice convulsing with the various aspartame doses was determined. $p < 0.05$, significantly different from 0 mg/kg as determined by the chi-square test.

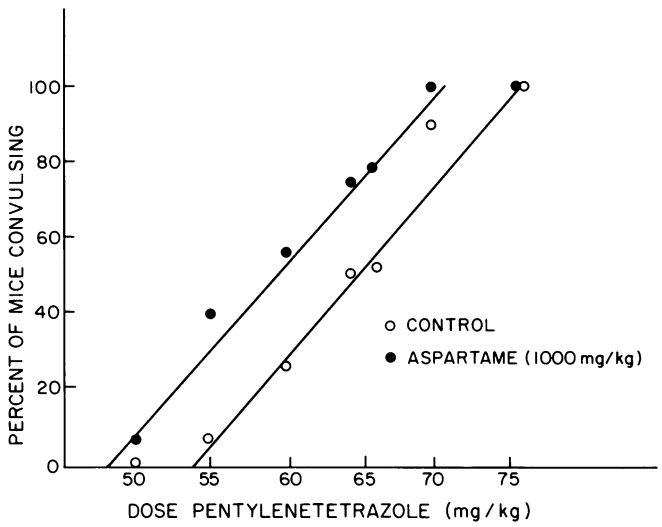

FIGURE 2. Effect of aspartame (1000 mg/kg) on the percentage of mice convulsing at various doses of pentylenetetrazole. Groups (average n = 24) or male CD-1 mice received water or 1000 mg/kg aspartame via oral intubation followed by various doses of pentylenetetrazole, 1 hr later. The number of animals convulsing was determined. Aspartame pretreatment significantly ($p < 0.05$) shifted the dose-response curve as determined by the method of Litchfield and Wilcoxon.

until a given animal had a seizure (when the treatment was inhaled fluorothyl or electroshock). The aspartame doses used were those shown, in the mice, to cause blood phenylalanine levels to rise by at least as much as blood tyrosine, i.e., doses of 1000 mg/kg or greater.

Aspartame administration produced a dose-dependent increase in seizure frequency among animals subsequently receiving the CD_{50} dose of pentylenetetrazole (PTZ) (65 mg/kg) (Fig. 1). At the 1000 and 2000 mg/kg aspartame doses, 78 and 100% of the animals experienced seizures, compared with 50% in the water-pretreated group (*26*). Other mice pretreated with a fixed dose (1000 mg/kg) of aspartame, or with water, and given various doses (50–75 mg/kg) of PTZ an hour later exhibited a significant leftward shift of the PTZ dose-response curve (Fig. 2). Enhanced susceptibility to PTZ-induced seizures was also observed among mice pretreated with phenylalanine (in doses equimolar to effective aspartame doses), but not among animals pretreated with aspartic acid or methanol. Coadministration with aspartame of the LNAA valine, which competes with phenylalanine for passage across the blood-brain barrier (*4,21*), protected mice from the seizure-promoting effects of the sweetener; in contrast, alanine, an amino acid which does not compete with phenylalanine for brain uptake, failed to attenuate aspartame's effect on PTZ-induced seizures.

A seizure-promoting effect of aspartame was also observed in mice developing seizures in response to fluorothyl or to electroshock. Animals were pretreated 60 min before exposure to 10% fluorothyl (delivered in a sealed chamber at a rate of 0.05 mL/min), and the time each took to develop clonus was measured. Water-pretreated control animals experienced clonus at 462 ± 18 sec, while those receiving aspartame (1000 mg/kg) experienced clonus 35% sooner (298 ± 10 sec) ($p < 0.001$) (*26*). This enhancement of seizure susceptibility was also mimicked by equimolar phenylalanine (but not aspartic acid) and blocked by valine, which, given alone, failed to alter the time to clonus. Aspartame (1000 mg/kg administered for 7 consecutive days) also accelerated the onset of hind limb flexion among mice given electroshock (50 mA, 60 Hz, 0.2 sec), a response that was mimicked by equimolar phenylalanine (Pinto and Maher, unpublished observations).

Discussion

The above data indicate that APM has seizure-promoting activity in animal models that are widely used to identify compounds affecting (i.e., usually protecting against) seizure incidence. That its mechanism of action involves increased brain phenylalanine is indicated by the ability of equimolar phenylalanine to simulate the epileptogenic effect and by the ability of concurrently administered valine to protect against this effect. The evidence does not indicate that aspartame itself causes seizures; rather it promotes seizures in animals that are already at risk (that is, animals treated with PTZ, fluorothyl, or electroshock). In a similar manner, it is possible that doses of the sweetener that cause a sufficient increase in brain phenylalanine might increase seizure frequency among susceptible humans, or might allow seizures to occur in people who are vulnerable but without prior episodes. Whether or not aspartame actually does promote seizures in susceptible humans will have to be explored in controlled clinical trials.

It is unfortunate but perhaps not surprising that questions about aspartame's phenylalanine-mediated neurologic effects arose after the sweetener was added to the food supply. New clinical data and the development of new hypotheses, based on laboratory research, can raise questions about any relatively new compound, even after that compound has passed all of the safety tests required at the time of its approval. What seems most important is that processes be developed for monitoring possible adverse reactions after food additives are placed in the food supply, and for continuing the conduct of government-mandated safety research. Perhaps experience with aspartame will cataylze the development of such processes.

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